Scientific Opinion on a request from the European Commission for the assessment of the scientific elements put forward by Hungary to support the prohibition for the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary

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Abstract: Hungary notified to the European Commission its scientific arguments justifying the implementation of a national safeguard measure prohibiting the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary, after which the European Commission asked the European Food Safety Authority (EFSA) to assess the scientific information supporting the prohibition. Having considered the information package provided by Hungary and all relevant scientific publications, the EFSA Panel on Genetically Modified Organisms (GMO Panel) concluded that (i) no new data specific to the safety of the nptII gene have been provided; (ii) the therapeutic relevance of kanamycin and neomycin was already addressed in the previous EFSA opinion on antibiotic resistance marker genes and kanamycin resistance in Mycobacterium tuberculosis results largely from chromosomal mutations and not from the transfer of aminoglycoside resistance genes such as nptII; (iii) the knowledge gaps and uncertainties highlighted in the Hungarian document have already been considered in the previous EFSA opinion on antibiotic resistance marker genes, and no new information on the safety of nptII gene as present in the GM potato EH92-527-1 has been identified in the scientific literature that would cause the GMO Panel to change its previous conclusions. Therefore, the EFSA GMO Panel concludes that no grounds exist to date that would lead to reconsideration of its opinion on GM potato EH92-527-1.

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SCIENTIFIC OPINION

Scientific Opinion on a request from the European Commission for the assessment of the scientific elements put forward by Hungary to support the prohibition for the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

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ABSTRACT

Hungary notified to the European Commission its scientific arguments justifying the implementation of a national safeguard measure prohibiting the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary, after which the European Commission asked the European Food Safety Authority (EFSA) to assess the scientific information supporting the prohibition. Having considered the information package provided by Hungary and all relevant scientific publications, the EFSA Panel on Genetically Modified Organisms (GMO Panel) concluded that (i) no new data specific to the safety of the *npt*II gene have been provided; (ii) the therapeutic relevance of kanamycin and neomycin was already addressed in the previous EFSA opinion on antibiotic resistance marker genes and kanamycin resistance in *Mycobacterium tuberculosis* results largely from chromosomal mutations and not from the transfer of aminoglycoside resistance genes such as *npt*II; (iii) the knowledge gaps and uncertainties highlighted in the Hungarian document have already been considered in the previous EFSA opinion on antibiotic resistance marker genes, and no new information on the safety of *npt*II gene as present in the GM potato EH92-527-1 has been identified in the scientific literature that would cause the GMO Panel to change its previous conclusions. Therefore, the EFSA GMO Panel concludes that no grounds exist to date that would lead to reconsideration of its opinion on GM potato EH92-527-1.

KEY WORDS

GMOs, potato *Solanum tuberosum*, EH92-527-1, Amflora, Hungary, safeguard clause, human and animal health, environment, Directive 2001/18/EC.

[1] On request from the European Commission, Question No EFSA-Q-2012-00615, adopted on 5 December 2012.

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SUMMARY

Following a request from the European Commission, the Panel on Genetically Modified Organisms (GMO) was asked to deliver a scientific opinion on the elements put forward by Hungary to support the prohibition for the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary.

In December 2010, Hungary notified to the EC its scientific argumentation justifying the implementation of a national safeguard measure prohibiting the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary, according to Article 23 of Directive 2001/18/EC on the deliberate release in the environment of genetically modified organisms.

On 23 May 2012, the European Food Safety Authority (EFSA) was requested by the European Commission to assess the scientific information submitted by the Hungarian Authorities in the context of a safeguard clause invoked under Article 23 of Directive 2001/18/EC.

In light of the information package provided by Hungary in support of its safeguard clause and, having considered all relevant scientific publications, the GMO Panel concludes that:

Hungary did not provide any new or additional information made available since the date of consent for this GM event that would affect the environmental risk assessment or the reassessment of existing information on the basis of new or additional scientific knowledge. New data specific to the safety of the *npt*II gene have not been provided.

The therapeutic relevance of kanamycin and neomycin was already addressed in the previous EFSA opinion on antibiotic resistance marker genes. Kanamycin resistance in *Mycobacterium tuberculosis* results largely from chromosomal mutations and not from the transfer of aminoglycoside resistance genes such as *npt*II.

The knowledge gaps and uncertainties highlighted in the Hungarian document have already been considered in the previous EFSA opinion on antibiotic resistance marker genes. EFSA continually reviews the scientific literature. No new information on the safety of the *npt*II gene, as present in the GM potato EH92-527-1, was identified in the scientific literature that would cause the GMO Panel to change its earlier conclusions.

The EFSA GMO Panel concludes that no detailed grounds exist to date that would lead to reconsideration of its opinion on GM potato EH92-527-1.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In February 2006, the European Food Safety Authority (EFSA) adopted an opinion related to the placing on the market of GM potato EH92-527-1 for cultivation and industrial starch production, following a notification submitted by BASF Plant Science to the Swedish Authorities.

On 11 June 2009, EFSA published a joint scientific opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ) on the "*Use of antibiotic resistance genes as marker genes in genetically modified plants*" and a scientific opinion of the GMO Panel on "*Consequences of the opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants on previous EFSA assessments of individual GM plants*".

On 2 March 2010, the European Commission (EC) adopted a decision authorising the placing on the market of GM potato EH92-527-1 for cultivation and industrial starch production.

In December 2010, Hungary notified to the EC its scientific argumentation justifying the implementation of a national safeguard measure prohibiting the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary, according to Article 23 of Directive 2001/18/EC on the deliberate release in the environment of genetically modified organisms.

In order for the EC to follow-up on this safeguard clause in accordance with Article 23 of Directive 2001/18/EC, it was deemed appropriate by EC that EFSA would assess the scientific elements provided by Hungary.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA was requested in accordance with Article 29 of Regulation (EC) No 178/2002 to assess the scientific information submitted by the Hungarian Authorities justifying their national safeguard measure concerning GM potato EH92-527-1 and to identify whether these new scientific elements might lead the GMO Panel to reconsider its opinion on GM potato EH92-527-1 from 2006.

ASSESSMENT

1. Introduction

Directive 2001/18/EC provides the possibility for the Member States to invoke safeguard measures on specific genetically modified organisms in the case where new or additional information, made available since the date of the consent, or reassessment of existing information on the basis of new or additional scientific knowledge would affect the risk assessment of an authorised GMO. Provisions foreseen by Hungary seek to provisionally prohibit the marketing of potato EH92-527-1 for cultivation purposes in Hungary.

The EFSA GMO Panel examined the set of supporting documents submitted by Hungary. In this respect, the GMO Panel assessed whether the submitted documents comprise new scientific information that would change the outcome of previously performed risk assessments, and if detailed grounds exist that would lead the GMO Panel to reconsider its opinion on GM potato EH92-527-1 (EFSA, 2006).

The GMO Panel looked for evidence of GMO-specific risks, taking into consideration the EFSA GMO Panel Guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011) as well as any related risk assessments carried out in the past. In addition, the GMO Panel considered the relevance of the concerns raised in the light of the most recent scientific data and relevant peer-reviewed publications regarding the use of specific antibiotic resistance genes as marker genes in GM plants.

2. Concerns raised by Hungary

The GMO Panel interprets the documentation provided by Hungary as raising the following issues:

- antibiotic resistance genes can be transferred from the food into the intestinal flora, and there is a high risk of gene transfer from the GM potato into the bacteria living in the intestines of animals (Section 3.1);

- gene transfer from cultivated GM potato plants in the soil bacteria escalates the multiplication of antibiotic resistance genes in the soil (Section 3.2);

- there is a knowledge gap regarding up-to-date data on the prevalence of *npt*II in soil environments throughout Europe (Section 3.3);

- there is a concern regarding the role of kanamycin and neomycin in relation to the treatment of tuberculosis and the possible appearance of resistance (Section 3.4).

3. Risk assessment of the *npt*II gene

3.1. Potential transfer of the *npt*II gene from GM potato into the intestinal flora

Hungary provided several references to support its claims that numerous examples show that the antibiotic resistance genes can be transferred from the food into the intestinal flora, and that there is a high risk of gene transfer from the GM potato into the bacteria living in the intestines of animals (Chowdhury *et al*., 2003; Mazza *et al*., 2005; Alexander *et al*., 2007; Chainark *et al*., 2008; Tudisco *et al*., 2010).

The review of Alexander *et al*. (2007) highlights an efficient degradation of DNA: no full-size transgenes were reported to be present in livestock. The review does not consider gene transfer from GM plants into the bacteria. Chainark *et al*. (2008) detected the presence of a recombinant CaMV 35S promoter fragment in the gastrointestinal tract content and blood (leucocytes) and tissue samples (kidney and muscle) of rainbow trout during feeding with GM soybean. Three to five days after

withdrawal of the GM feed, it was no longer detected. The study did not investigate the intestinal microbiota. The purpose of the study carried out by Chowdhury *et al*. (2003) was to determine if recombinant *cry1Ab* gene could be detected in the gastrointestinal contents of pigs fed GM maize. Fragments of the gene were detected in stomach, duodenal, ileal, caecal and rectal contents, but no evidence was provided for the transfer of the gene to the intestinal flora. The goal of the work of Mazza *et al*. (2005) was to assess the persistence of feed-derived *cry1Ab* fragments in the tissues (blood, spleen, liver, kidney, muscle) of piglets. Only a small fragment was detected, not the intact gene or its minimal functional unit. The intestinal microbiota was not analysed. Tudisco *et al*. (2010) analysed the presence of transgene fragments in blood and milk of goats fed GM soybean. The intestinal microbiota was not investigated.

The GMO Panel assessed the literature cited in support of the Hungarian claims and concluded that no evidence was provided by Hungary to support the claim of a high risk of gene transfer from the GM potato into the intestinal flora of the animals. The GMO Panel has acknowledged this issue previously and assessed its implications (EFSA, 2009). There is no new information, either in the documentation submitted by Hungary or in the scientific literature, that would cause the Panel to change its former conclusions. Therefore, the GMO Panel reiterates its conclusion that, taking into account all the limitations of all current methodologies of detection, it can be expected that there is, at most, a low probability of transfer of antibiotic resistance genes from GM plants to bacteria in the digestive tract of humans and animals. If this transfer were to occur, it would take place at an extremely low frequency. In the risk assessment, the GMO Panel took into account the subsequent development and dissemination of resistance among bacteria.

Furthermore, Hungary claims that, because of the "high-speed processing machines" used during potato harvesting, transgenic DNA might easily be exposed and the probability of horizontal gene transfer is much higher in animal (ruminant in particular) intestinal bacterial flora than in the case of intact cells and plant material (Chowdhury *et al*., 2003; Netherwood *et al*., 2004). The references provided do not support such claims.

3.2. Potential transfer of the *npt*II gene from GM potato in the soil bacteria and between bacteria

Hungary provides references meant to support its claim that there is a high risk of gene transfer from the GM potato in the soil bacteria (Martinez, 2009; Allen *et al*., 2010; Knapp *et al*., 2010), and that the production of GM plants escalates the multiplication of antibiotic resistance genes in the soil (Allen *et al*., 2010).

The review of Allen *et al*. (2010) deals with antibiotic resistance genes in natural environments. Gene transfer from GM plants into the soil bacteria is not discussed, nor is there any indication that the production of GM plants escalates the multiplication of antibiotic resistance genes in the soil. Knapp *et al*. (2010) studied historical soil samples (from 1940 to 2008) from different locations in the Netherlands and concluded that antibiotic resistance genes from all classes of antibiotics tested (*npt*II or related genes were not studied) have significantly increased since 1940. It should be noted that this is not evidence for gene transfer from GM potato to soil bacteria. The review of Martinez (2009) discusses the possibilities of human pathogenic bacteria acquiring antibiotic resistance genes, e.g., from environmental microbes. Gene transfer from GM plants into soil bacteria is not discussed. In conclusion, no evidence was provided to support the claim of a high risk of gene transfer from the GM potato into the soil bacteria.

Regarding the EFSA (2009) statement that antibiotic resistance occurs in nature in soil bacteria, Hungary provides literature to support the claims that the bacteria infecting animals and humans are not soil bacteria and that resistance is not frequent in these pathogens (Heinemann, 1999; Heinemann *et al*., 2000). Both reviews have a therapeutic focus, dealing with drug resistance in pathogens and the problem of antimicrobial drug design. They do not consider resistance frequencies or the differentiation between soil bacteria and human pathogenic bacteria. On the other hand, Hungary

provides literature to support the claim that the human and animal intestines facilitate the inter-bacterial gene transfer and transfer from food-ingested bacteria into intestinal bacteria (Ferguson *et al*., 2002; Hehemann *et al*., 2010). Ferguson *et al*. (2002) demonstrated gene transfer between *Salmonella enterica* serovar Typhimurium by conjugation inside cultured human epithelial cells. Hehemann *et al*. (2010) provided evidence that intestinal flora can acquire new genes from microbes living outside the gut, e.g., from seaweed-associated marine bacteria. Both examples deal with gene transfers between bacteria, and as such are not evidence for the transfer of genes from GM plants to microbes.

Hungary concluded that there are no reliable data to document the likelihood of transfer of the *npt*II gene from GM plant to the bacterial flora. The GMO Panel acknowledged (EFSA, 2009) that there are uncertainties regarding the extent of horizontal gene transfer from plant DNA to bacteria in natural conditions (Nielsen *et al*., 2005), and regarding the impact that such transfers, compared with gene exchange, would have on bacterial populations.

The GMO Panel further acknowledged (EFSA, 2009) the limitations in the screening of DNA uptake in natural bacterial communities (e.g., in soil or in the gut), including the limited ability to prove the uptake of DNA in the unculturable fraction, limited focus on anaerobic bacteria, a highly limited coverage of locations and time points, and the limited attention given to selection in driving the population dynamics of rare transformants (Heinemann and Traavik, 2004; Nielsen and Townsend, 2004).

The EFSA GMO Panel acknowledges that the distribution of *npt*II in naturally occurring bacteria may provide opportunities for transfer and recombination of this gene, or fragments thereof, among bacteria. Transfer of *npt*II among bacteria can occur by transformation, conjugation or transduction. Horizontal gene transfer from plant to bacteria can occur only by transformation and has only been shown to occur with plant DNA that has sequence similarity with the bacterial DNA. The EFSA GMO Panel therefore stresses that transfer of the *npt*II gene from plant to bacteria would be expected to occur with a frequency several orders of magnitude lower than the frequency obtained by transfer of DNA between bacteria (EFSA, 2009).

There is no new information, either in the documentation submitted by Hungary or in the scientific literature, that would cause the Panel to change its previous conclusions. Therefore, the GMO Panel reiterates its conclusion that, taking into account all the limitations of all current methodologies of detection, it can be assumed that there is, at most, a low probability of transfer of antibiotic resistance genes from GM plants to bacteria in the environment. If this transfer were to occur, it would take place at an extremely low frequency. In the risk assessment, the GMO Panel took into account the consequential development and dissemination of resistance between bacteria if such transfer of antibiotic resistance would occur.

3.3. Quantitative data on the occurrence of *npt*II in natural habitats

The EFSA GMO Panel agrees with the Hungarian Authorities that there is a knowledge gap regarding up-to-date data on the prevalence of *npt*II in soil environments throughout Europe. The *npt*II gene occurs in bacteria at different frequencies in different species, isolates and environments (EFSA, 2009).

Recent metagenomic analyses of total bacterial populations (including non-culturable bacteria) have demonstrated that various types of resistance determinants, including resistance determinants for kanamycin and neomycin, have been detected in all the environments investigated (reviewed in EFSA, 2009; Allen *et al*., 2010). These investigations indicate that aminoglycoside resistance determinants have a wide distribution, albeit at different frequencies, in different species, isolates and different environments, in naturally occurring bacteria.

The EFSA GMO Panel points out that the use of antibiotics is a key factor in the selection and dissemination of antibiotic resistance genes in the immediate environment. While the key role of selection by antibiotic usage in the development of resistance is indisputable, some knowledge gaps

remain regarding the understanding of the natural reservoir of antibiotic resistance genes and their role in natural bacterial communities not exposed to industrially produced antibiotics, as previously concluded (EFSA, 2009).

3.4. Multidrug-resistant tuberculosis and kanamycin

The therapeutic relevance of kanamycin and neomycin was already addressed in the EFSA's opinion on antibiotic resistance marker (ARM) genes (EFSA, 2009). Kanamycin is used as a second-line drug for the treatment of multiple drug-resistant tuberculosis (MTB). Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) (XDR-TB) are reported to have gradually spread across European countries with low TB prevalence (Cohen-Bacrie *et al*., 2011). By the end of 2010, 68 countries, including 19 of the 27 countries in the EU, had reported at least one XDR-TB case (WHO, 2010). The increasing occurrence worldwide of TB isolates with resistance to second-line antibiotics such as kanamycin is a cause for global concern.

It should be noted, however, that kanamycin resistance in the XDR-TB strains largely results from mutations in the 16S ribosomal RNA gene, the promoter of the enhanced intracellular survival gene (*eis*) and the glucose-inhibited division protein B gene (*gidB*). Thus, resistance to kanamycin in such strains is chromosomally encoded and has not developed as a result of the transfer of aminoglycoside resistance genes, such as *npt*II (Johnson *et al*., 2006; Zaunbrecher *et al*., 2009; Georghiou *et al*., 2012).

CONCLUSIONS

The EFSA GMO Panel examined the document submitted by Hungary. The Panel assessed whether the document contained new scientific information and concludes that:

Hungary did not provide any new or additional information made available since the date of consent for this GM event that would affect the environmental risk assessment or the reassessment of existing information on the basis of new or additional scientific knowledge[4]. New data specific to the safety of the *npt*II gene have not been provided.

The therapeutic relevance of kanamycin and neomycin was already addressed in the previous EFSA opinion on antibiotic resistance marker genes. Kanamycin resistance in *Mycobacterium tuberculosis* results largely from chromosomal mutations and not from the transfer of aminoglycoside resistance genes such as *npt*II.

The knowledge gaps and uncertainties highlighted in the Hungarian document have already been considered in the previous EFSA opinion on antibiotic resistance marker genes. EFSA continually reviews the scientific literature. No new information on the safety of the *npt*II gene, as present in the GM potato EH92-527-1, was identified in the scientific literature that would cause the GMO Panel to change its earlier conclusions.

The EFSA GMO Panel concludes that no detailed grounds exist to date that would lead to reconsideration of its opinion on GM potato EH92-527-1.

DOCUMENTATION PROVIDED TO EFSA

1. Letter, received 23 May 2012, with supporting document from Ladislav Miko, Deputy Director-General for the food chain EC, to Catherine Geslain-Lanéelle, Executive Director EFSA (ref. SANCO/E1/MD/mp Ares(2012) 623655), requesting the assessment by EFSA of the scientific elements provided by Hungary in support of its decision to implement a national safeguard measure under Article 23 of Directive 2001/18/EC for GM potato EH92-527-1 and comprising the following supporting document:

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities L106: 1-38 (Article 23).

- Scientific and other proof relating to the Hungarian safety measure taken as regards the genetically modified Amflora potato.

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